



Docket No. 0508-1053-1
Appln. No. 10/776,330

AMENDMENTS TO THE CLAIMS:

This listing of claims will replace all prior versions, and listings, of claims in the application:

LISTING OF CLAIMS:

1. (Currently Amended) A process for detecting and/or quantifying non-covalent interactions between a target [(protein)] receptor and one of its ligands, comprising:

- preparing cells or cell fragments containing a nucleic acid sequence [(expressing)] encoding a fluorescent protein, fused with a nucleic acid sequence encoding the target [(protein)] receptor, the fusion between the nucleic acid sequence for the fluorescent protein and the [(gene)] nucleic acid sequence for the target [(protein)] receptor being so that the properties of the target [(protein)] receptor are not modified by the presence of the fluorescent protein:

* wherein the interaction between the target [(protein)] receptor, and the ligand is not modified, and

* wherein a response transduction function is not modified,

the fluorescent protein is selected from Green [(fluorescent)] Fluorescent [(proteins)] Proteins (GFPs) obtained or derived from autofluorescent proteins of [(cnidarians)] Cnidarians, the molar extinction coefficient of which is greater than about $14,000 \text{ M}^{-1}\text{cm}^{-1}$ and the [(quantic)] quantum fluorescence yield is greater than about 0.38, ~~this protein being selected from the group consisting of green fluorescent protein (GFP),~~;

- placing said cells or said cell fragments in contact with a ligand for said target [(protein)] receptor, said ligand labeled with a [(molecule)] label capable of absorbing the

light emitted by the fluorescent protein, or a fluorescent substance, the fluorescent protein being the fluorescence energy donor and the label being the fluorescence energy acceptor, or the fluorescent protein being the fluorescence energy acceptor and the label being a fluorescent substance which is a fluorescence energy donor[,]; and

- irradiating said cells or said cell fragments at a wavelength which makes it possible either to excite the fluorescent protein or to excite the [(fluorescent substance)] label,

- wherein the steps of placing in contact and irradiating are carried out either simultaneously or one after the other, or

- said cells or said cell fragments are placed in contact with a ligand for said [(protein)] target receptor, said ligand labeled with a label, the cells or the ligand having been irradiated before being placed in contact,

- wherein a reduction in the amplitude of the donor's emission and/or emission signal characteristic of the acceptor's emission is measured and measuring the fluorescence energy transfer to detect and/or quantify the non-covalent interactions.

2. (Currently Amended) The process according to Claim 1, wherein the [(protein)] target receptor ~~whose protein-ligand interaction~~ is selected from the group consisting of:

-membrane-bound [(proteins)] G protein coupled receptors (GPCRs) ~~coupled to the G protein~~,

-insuline-like growth factor (IGF) receptors ~~which are structurally linked to the insulin receptor~~,

-ion channel-receptors, and

-intracellular nuclear receptors which are structurally linked to the steroid receptor.

3. (Currently Amended) The process according to Claim 1, wherein the fluorescent protein is [(Enhanced Green Fluorescent)] enhanced green fluorescent protein (EGFP) and the labeled substance is Bodipy and in which either the reduction in the emissions amplitude of EGFP or an emission signal of Bodipy resulting from an energy transfer is detected, the irradiation wavelength corresponding to the excitation wavelength of EGFP.

4. (Currently Amended) The process according to Claim 1, wherein the fluorescent protein is [(Enhanced Green Fluorescent)] enhanced green fluorescent protein (EGFP) and the labelled substance is coumarin, and wherein either the diminution of amplitude of coumarin or an emission signal EGFP resulting from an energy transfer is detected, said irradiation wavelength corresponding to the excitation wavelength of coumarin.

5. (Currently Amended) The process according to Claim 1, wherein the fluorescent protein is fused on [(the)] its N-terminal side [(and)] to the C-terminal side of the target [(protein)] receptor is fused on the C-terminal side.

6. (Currently Amended) The process according to Claim 1, wherein the fluorescent protein is fused on [(the)] its C-terminal side [(and)] to the N-terminal side of the target [(protein)] receptor is fused on the N-terminal side.

7. (Currently Amended) The process according to Claim 1, wherein the fluorescent protein is inserted into a ~~receptor~~ target [(protein)] G protein coupled receptor (GPCR) ~~wherein said receptor is coupled to the G protein~~, this insertion taking place in the first or third intracellular loop of the receptor, with the proviso that the insertion does not destroy either the properties of the receptor or the fluorescence of the fluorescent protein.

8. (Currently Amended) The process according to Claim 1, wherein the cells are mammalian cells, which are adherent or in suspension selected from the group consisting of HEK 293 cells, CHO cells, COS cells, lymphocytic lines, fibroblasts, yeast cells selected from [(pichia)] Pichia pastoris, [(saccharomyces)] Saccharomyces cerevisiae, [(saccharomyces)] Saccharomyces kluyveri, Hansenula polymorpha, and insect cells.

9. (Currently Amended) The process according to Claim 1, wherein a signal can be detected after mixing the compound being a fluorescence energy donor and the compound being a fluorescence energy acceptor, and can be abolished by the addition of a non-fluorescent substance [(of similar pharmacological specificity)] having the same binding site, and wherein a signal/noise [(radio)] ratio is greater than about 2.

10. (Currently Amended) A process for detecting and/or quantifying non-covalent interactions between a target [(protein comprising a)] G protein coupled receptor coupled to G proteins and a G protein, in order to identify [(molecules)] compounds which are biologically active with respect to the

receptor, and which are capable of forming a reversible non-covalent interaction with said receptor, wherein:

- cells or fragments of cells are prepared [(which express)] containing a [(DNA)] nucleic acid sequence encoding for a fluorescent protein and fused with a nucleic acid [(expressing)] encoding for a receptor coupled to the G proteins are prepared, the fusion between the nucleic acid encoding for the fluorescent protein and the nucleic acid encoding for said receptor being so that the properties of the receptor are not modified by the presence of the fluorescent protein, namely: wherein,

- * the interaction between the target receptor and the G protein is not modified,
- * the interaction between the target receptor and the biologically active molecule is not modified,
- * a response transduction function is not modified,
- * the fluorescent protein is selected from [[the]] Green [(fluorescent)] Fluorescent [(proteins)] Proteins (GFPs) obtained or derived from autofluorescent proteins of [(cnidarians)] Cnidarians, the [(molecular)] molar extinction coefficient of which is greater than about 14,000 M⁻¹cm⁻¹ and the [(quantic)] quantum fluorescence yield of which is greater than about 0.38, this protein being a
green fluorescent protein (GFP),
wherein the G protein is labeled with a label [(comprising)] selected from:
 - [(- either a molecule which is capable of absorbing the light emitted by the selected fluorescent protein,
- or a [(fluorescent substance)) label)]

- Green Fluorescent Proteins (GFPs) obtained or derived from autofluorescent proteins of Cnidarians, the molar extinction coefficient of which is greater than about $14,000 \text{ M}^{-1}\text{cm}^{-1}$ and the quantum fluorescence yield is greater than about 0.38,
- fluorescent chemical compounds, and
- non-fluorescent chemical compounds belonging to the Acid Violet group, Acid Red group, alizarins, alumino, azocarmines, basic fuchsin, Bordeaux R and Carmine.

wherein the fluorescent protein and said label being such that they transfer energy from one to the other, wherein the fluorescent protein is an energy donor or ~~it~~ said label is an energy donor,

detecting the interaction between the target receptor labeled with the fluorescent protein and the G protein labeled with said label by fluorescence energy transfer and measuring the fluorescence energy transfer when quantifying the non-covalent interactions.

11. (canceled)

12. (Currently Amended) A kit or equipment for detecting and/or quantifying non-covalent interactions between a target [(protein)] receptor labeled with a fluorescent protein and one of its ligands labeled with a label [(consisting)], said kit comprising:

- ~~— a molecule which is capable of absorbing the light emitted by the fluorescent protein,~~
- ~~— or a fluorescent substance,~~

- [(this)] a fluorescent protein is selected from Green
[(fluorescent)] Fluorescent [(proteins)] Proteins (GFPs)
obtained or derived from autofluorescent proteins of
[(cnidarians)] Cnidarians, the [(molecular)] molar extinction
coefficient of which is greater than about 14,000 M⁻¹cm⁻¹ and
the [(quantic)] quantum fluorescence yield of which is greater
than about 0.38, ~~this protein being further chosen from green~~
~~fluorescent protein (GFP), that conserve the fluorescence~~
~~property~~

- the label for the ligand being selected from the
group consisting of:

- Green Fluorescent Proteins (GFPs) obtained or
derived from autofluorescent proteins of Cnidarians,
the molar extinction coefficient of which is greater
than about 14,000 M⁻¹cm⁻¹ and the quantum fluorescence
yield is greater than about 0.38,
- fluorescent chemical compounds (eg. Bodipy or
coumarin), and
- non-fluorescent chemical compounds belonging to
the Acid Violet group, Acid Red group, alizarins,
aluminon, azocarmines, basic fuchsin, Bordeaux R and
Carmine,

~~and its ligand labeled with a fluorescent substance, the said~~
~~kit comprising:~~

- the target [(protein)] receptor fused with a
fluorescent protein or a stable cell line which is capable of
expressing the [(protein)] target receptor fused with a
fluorescent protein or a plasmid containing the nucleic acid
sequence coding for said target [(protein)] receptor fused with
a fluorescent protein as defined above, and

- the ligand labeled with [(said)] selected label,

~~— buffers and media required for the energy transfer between said protein and said ligand.~~

13. (Currently Amended) A kit or equipment for detecting and/or quantifying non-covalent interactions between a target [(protein)] receptor labeled with a first fluorescent protein and one of its ligands labeled with a [(fluorescent substance)] label corresponding to a second fluorescent protein, said first fluorescent protein being chosen from ~~the fluorescent protein~~ enhanced yellow fluorescent protein (EYFP) or enhanced green fluorescent protein (EGFP) and the ligand being labeled with [(said)] a second fluorescent protein being enhanced cyan fluorescent protein (ECFP), or said first fluorescent protein being ECFP and the ligand being labeled with ~~said second fluorescent protein~~ EYFP or EGFP, said kit comprising:

- a plasmid containing a nucleic acid sequence coding for the target [(protein)] receptor fused with a fluorescent protein, and a plasmid containing a nucleic acid sequence coding for the ligand fused with a second fluorescent protein, or a ligand fused with a second fluorescent protein, obtained via a recombinant route and purified, or

- a stable cell line which is capable of expressing the target [(protein)] receptor fused with first fluorescent protein, and a stable cell line which is capable of expressing the ligand fused with a second fluorescent protein or a ligand fused with [(a)] the selected second fluorescent protein[(,)].
~~obtained via a recombinant route and purified, and~~

~~— buffers and media required for an energy transfer between said protein and the said ligand.~~

14. (Currently Amended) A kit or equipment for detecting and/or quantifying non-covalent interactions between a target [(protein)] receptor consisting of a G protein coupled receptor ~~coupled to the G protein~~ labeled with a first fluorescent protein and a G protein labeled with a fluorescent substance corresponding to a second fluorescent protein, the first fluorescent protein being chosen from [(the)] enhanced yellow fluorescent protein (EYFP) or enhanced green fluorescent protein (EGFP) and the G protein being labeled with the second fluorescent protein being enhanced cyan fluorescent protein (ECFP) or the first fluorescent protein being ECFP and the G protein being labeled with the second fluorescent protein being selected from EYFP or EGFP, said kit comprising:

- a plasmid containing a nucleic acid sequence coding for the receptor fused with [(a)] the selected first fluorescent protein, and a plasmid containing a nucleic acid sequence coding for the G protein fused with [(a)] the selected second fluorescent protein, or the G protein fused with [(a)] the selected second fluorescent protein, obtained via a recombinant route and purified, or

- a stable cell line which is capable of expressing the receptor fused with [(a)] the selected first fluorescent protein, and a stable cell line which is capable of expressing the G protein fused with [(a)] the selected second fluorescent protein, or the G protein fused with [(a)] the selected second fluorescent protein second, obtained via a recombinant route and purified[(, and)].

~~—buffers and media required for an energy transfer between the above-mentioned receptor and the above-mentioned G protein.~~

15-16. (canceled)

17. (new) The process according to claim 1, wherein the label for the ligand is selected from:

- Green Fluorescent Proteins (GFPs) obtained or derived from autofluorescent proteins of Cnidarians, the molar extinction coefficient of which is greater than about 14,000 M⁻¹cm⁻¹ and the quantum fluorescence yield is greater than about 0.38,

- fluorescent chemical compounds, and
- non-fluorescent chemical compounds belonging to the Acid Violet group, Acid Red group, alizarins, aluminon, azocarmines, basic fuchsin, Bordeaux R and Carmine.

18. (new) The process according to Claim 1, wherein the fluorescent protein is selected from: green fluorescent protein (GFP) or enhanced green fluorescent protein (EGFP), cyan fluorescent protein (CFP) or enhanced cyan fluorescent protein (ECFP), yellow fluorescent protein (YFP) or enhanced yellow fluorescent protein (EYFP), and green fluorescent protein UV (GFPUV).

19. (new) A process for detecting and/or quantifying non-covalent interactions between a target receptor and one of its ligands, comprising:

providing a fluorescent protein fused with a target receptor of a cell, wherein,

the fusion does not affect either the interaction between said target receptor and a ligand for said receptor or a response transduction function, and

said fluorescent protein is a Green Fluorescent Protein obtained or derived from autofluorescent proteins of Cnidarians having a molar extinction coefficient greater than about 14,000 M⁻¹cm⁻¹ and a quantum fluorescence yield greater than about 0.38;

placing a ligand for said target receptor in contact with said fluorescent protein fused with said target receptor, wherein,

said ligand is labeled with a label capable of absorbing light emitted by said fluorescent protein, and

said fluorescent protein is a fluorescence energy donor and said label is a fluorescence energy acceptor, or said fluorescent protein is a fluorescence energy acceptor and said label is a fluorescent substance that is a fluorescence energy donor;

irradiating the fluorescence energy acceptor and/or fluorescence energy donor at a wavelength to provide a measurable emission signal; and

measuring the amplitude of the donor's emission and/or emission signal characteristic of the acceptor's emission and the

fluorescence energy transfer in order to detect and/or quantify the non-covalent interactions.

20. (new) The method according to claim 19, further comprising:

preparing a cell or cell fragment containing a nucleic acid sequence encoding a fluorescent protein fused with a nucleic acid sequence encoding a target receptor of a cell to produce said fluorescent protein fused with a target receptor of a cell.

21. (new) The method according to claim 20, wherein said placing a ligand for said target receptor in contact with said fluorescent protein fused with said target receptor comprises contacting said ligand with said cell or said cell fragment.

22. (new) The method according to claim 19, wherein said target receptor of a cell is selected from the group consisting of membrane-bound G protein coupled receptors, insulin-like growth factor receptors, ion channel-receptors, and intracellular nuclear receptors.